

CAR-T cells therapy: a potential new strategy against prostate cancer

Shanglin Yang *

Department of Bioengineering, East China University of Science and Technology, Shanghai, China

* Corresponding author: 20000583@mail.ecust.edu.cn

Abstract. There have already been some therapies for cancer, but further research is still in urgent need to effectively treat prostate cancer. Chimeric antigen receptor T (CAR-T) cells therapy is a popular new therapy to treat tumor, although it is mostly in the clinical research stage except for a few specific applications. Prostate cancer is a type of malignancy tumor in man occurring frequently. It has the second highest incidence in man and the fifth highest fatality rate worldwide. At present, the main treatment for prostate cancer includes reduce the androgen concentration of patients through drugs, operation to remove prostate tumor, pelvic radio therapy and chemotherapy. On how CAR-T works to treat prostate cancer, more study is currently being done. This review introduces the structure and the feasibility of CAR-T as a means of treating prostate cancer due to the research of three antigens which have prostate specific (prostate specific membrane antigen, prostate stem cell antigen and epithelial cell adhesion molecule) and discusses their application in prostate cancer.

Keywords: Prostate Cancer, CAR-T, Structure of CAR-T, PSMA, PSCA, EpCAM.

1. Introduction

In 2020, based on the data of cancer incidence and mortality from GLOBOCAN 2020 which International Agency for Research on Cancer published, it is estimated that there are 1.4 million newly confirmed cases of prostate cancer and 375000 deaths worldwide. Prostate cancer is a kind of malignancy tumor in man occurring frequently. It has the second highest incidence in man and the fifth highest fatality rate worldwide. In almost half of the countries, prostate cancer is a cancer which is the most common malignant tumor in men [1]. In addition, accompanied by high incidence and mortality, there will also be various complications caused by prostate cancer, with the growth of tumor. These complications are mainly manifested as the impact on male urinary system and reproductive system functions. Prostate cancer is a malignant solid tumor and traditional cancer therapy cannot effectively treat prostate cancer in the advanced or terminal stage with the unnoticeable early symptoms associated with it. There are many factors causing prostate cancer, such as diet, smoking and overweight and the probability of getting prostate cancer will increase with age. These data show that prostate cancer is a serious malignant tumor for men.

Cancer cells can cause immune suppression by activating different immune checkpoint pathways which makes cancer cells harder to kill. Great breakthrough has been made in cancer treatment, relying on monoclonal antibodies targeting immune checkpoints and immune checkpoint inhibitors (ICIs) is a therapy of treating cancer based on this method. ICIs can block the co inhibition signal pathways to reactivate the immune response which can search for and kill cancer cells and are also beneficial to the elimination of cancer cells mediated by immunity. Among the ICIs, good therapeutic effect on cancer has been showed by programmed death-1/programmed cell death-ligand 1 (PD-1/PD-L1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) inhibitors and some of inhibitors have been used in some practical cancer treatments, meanwhile there are many other ICIs in clinical trials, but there are still some problems in ICIs therapy. The mainstream inhibition of ICIs therapy for cancer is influenced by adverse events which is caused by the immune system and rate of response [2]. Although PD-1/PD-L1 and CTLA-4 inhibitor have made some achievements in the treatment of cancer, few cancer patients benefit from ICIs therapy that the progression of their tumors was slowed, and several of those getting it experienced substantial immune-related side effects (irAEs) [3]. irAEs are immune activation caused by adjusting the activity of T cells, which can cause adverse reactions

related to immune system, thus causing related symptoms of corresponding organs. Using ICIs that identify CTLA-4 and PD-1/PDL-1 as targets produces a series of unique toxic reactions caused by T cell tissue infiltration, because of increased immune activity or excessive immune activation.

CAR-T is a new targeted cancer therapy. It has the characteristics of accuracy, speed and efficiency, and is a new tumor immune therapy with the possibility of curing cancer. After optimization and improvement in recent years, in the clinical therapy of tumors, it has produced excellent outcomes and it has a good development prospect. The patient's own T cells are obtained for the therapy and deliver a virus vector carrying chimeric antigen receptor which can activate T cells and tumor cells can be identified by it to transform T cells into CAR-T cells. After multiply in vitro, the patient will inject CAR-T cells and cells can effectively kill tumor cells to cure the cancer. At present, applications of CAR-T mainly concentrate on treatment of lymphoma. Acute B-lymphocyte leukemia is a common acute lymphocytic leukemia which is a malignant tumor often happening to children and teenagers. Although past therapies can work on it, they are hard for recurrent acute B-lymphocyte leukemia and refractory acute B-lymphocyte leukemia to have therapeutic effect. Most patients can only get short-term disease control after receiving treatment, and the survival period is also not long, but the appearance of CAR-T has greatly changed this situation and CAR-T has greatly extended the survival period of patients.

2. Structure of CAR-T

2.1. Four parts of CAR-T

The structure of CAR-T is mainly consisted of four parts including an intracellular domain, a transmembrane domain, a hinge region and an extracellular domain. The most usual ingredients of intracellular domain are CD3 ζ Including three immune receptor tyrosine group activation motifs [4]. The function of intracellular domain is co-stimulating molecules and activates intracellular signals to enable T cells to multiply and release cytokines. It can make the T cells more efficient at killing cancer cells. And this domain also has function about signal transduction. The closest component to the cell membrane in the cytoplasmic region is the transmembrane domain, and is consisted of a transmembrane hydrophobic alpha helix and amino acid sequences from CD4 is a common source of transmembrane domain [4, 5]. The domain links intracellular domain and extracellular domain of CAR-T cell. Besides, the receptor was anchored on the cell membrane by it. The most important role of hinge region is as a connection between antigen recognition domain and transmembrane domain. More critically, different choices of hinge may affect the function of CAR-T because they have different length and component of hinge region. Adaptability, CAR expression, signaling, activation outputs potency, and epitope recognition are affected by it, such as when antigen cannot be approached, using longer hinge region to overcome steric hindrance and more effectively combine with antigen [6,7]. Extracellular domain is the antigen binding domain and it can recognize TAA (tumor-associated antigen) Antigen binding domain is always derived from scFv (Single Chain Variable Fragment) whose main component is the variable heavy and variable light linked by a adaptability short peptide chain composed of 10-25 amino acids and is a smaller recombinant antibody. It not only retains the specificity of the original immunoglobulin to the antigen, but also enhances the therapeutic effect on tumors.

2.2. History: generations of structural and functional changes

In order to better improve the therapeutic effect on tumors and decrease adverse reactions in the body, CAR-T has experienced four generations of technological development for 30 years. The design structure of first generation who's the most obvious characteristic is single structure derived from CD3 ζ chain was relatively simple [8]. Full activation of T cell not only depends on the signal transmitted by the combination of extracellular antigen binding domain, but also relies on the combination of the co-stimulatory molecule receptors and their ligands to transmit signal. If there isn't co-stimulatory signal, T cells cannot function normally, even if the antigen stimulate T cells, but

the expression of co-stimulatory receptors on the surface of tumor cells is rarely detected. This problem was not considered when designing first generation and first generation lacked necessary co-stimulatory molecule receptors, so that it cannot fully activate CAR-T cells causing poor proliferation, cytotoxicity and life in vivo and its effect in clinical trials is not ideal. The transmission of two different signals is the condition for full T cell activation and to meet this condition, based on first generation, second generation added the intracellular signal domain which contained co-stimulatory molecule receptors, such as CD28. Proliferation and survival for T cells is heavily influenced by co-stimulatory of CD28 and it is closely related to memory cells and effector cells about their development [9]. In clinical trials, second generation significantly improves the activation of CAR-T immune activity and improves the duration of action. Third generation added another signal domain (4-1BB or OX40) on the basis that second generation has an original signal domain to enhance the signal transmission efficiency. However, this idea seemed reasonable, but in fact there were some problems, as second generation had better signal transduction than third generation reported by Moffitt Cancer Center [10]. Meanwhile, the third generation may reduce threshold of cell stimulation causing signal leakage and induce excessive cytokines release [11]. TRUCKs (T cell redirected for universal cytokine killing) is another name for fourth generation CAR-T cells which add a nuclear factor of the activated T cell signal transducer based on second generation and the chimeric receptor releases a number of cytokines stimulated by tumor antigens to modify the tumor micro environment so that it can collect and activate other immune cells for immune response [12]. A breakthrough in the treatment of solid tumors to a certain extent in CAR-T is made by it, but it needs further verification in clinical experiments.

3. CAR-T therapies in prostate cancer T

3.1. Prostate specific membrane antigen

Prostate specific membrane antigen (PSMA) is a type II membrane protein composed of 750 amino acids. It can make the internalization of proteins on the cell surface and turns them into endoplasmic compound, meanwhile, because of this specific feature, it become a possibility to be used as an antigenic target in therapy [13]. PSMA plays a crucial part in targeted therapy of prostate cancer as a therapeutic target and its overexpression appears on most prostate cancer cell surfaces, and this value will be higher in advanced and castration resistant prostate cancer patients. In CAR-T therapy, designed T cells against PSMA selectively target anti-expressing cells can kill tumor cells in the body and when its effect is enough to provide the ability to resist tumor cells, it does not show other harmful toxicity, which proves its safety [14]. In a clinical trial of TGF (transforming growth factor)- β -resistant CAR-T, 18 patients participated in the trial and thirteen participants received four doses of treatment. 5 of these patients showed cytokine release syndrome (CRS) of grade 2 or higher and one of five patients experiencing an obvious marked CAR-T cell expansion reduced prostate-specific antigen (PSA) by more than 98%, death following grade 4 CRS with concurrent sepsis, which the treatment effect is the most obvious. Three other patients showed >30% reduction in PSA [15]. The trial showed TGF- β -resistant CAR-T has a great therapeutic effect on a patient and also showed good efficacy in other three patients. It made PSMA to have the feasibility to be used in CAR-T and in most cases, it is safe and provided a broader idea for future research on the treatment of prostate cancer.

3.2. Prostate stem cell antigen

Prostate stem cell antigen (PSCA) is a glycoprotein containing 123 amino acids on the cell surface and PSCA mRNA expresses high prostate specificity in normal male tissues and in most prostate cancer can find that PSCA is highly expressed. In Reiter et al.'s study, 111 of 126 prostate cancer samples analyzed showed moderate to strong expression, accounting for 86% of the samples [16]. PSCA was located in chromosome region 8q24.2 where most advanced and relapsed prostate cancers have allele acquisition or amplification in this region by physical mapping and gene c2myc closely

related to primary prostate cancer and metastatic prostate cancer was also located at the proximal end of chromosome 8q24 [17]. All of these studies showed the possibility of using it as a target antigen for prostate cancer treatment. In animal trials, its monoclonal antibody has obvious effect on the growth of prostate cancer whether it is dependent on androgens or not. In a trial, to androgen dependent LAPC29 and androgen independent PC23 tumor bearing mice, both of two kinds of PSCA monoclonal antibody effectively inhibited the tumor and the survival period of tumor bearing mice was significantly prolonged by it [18]. But the action mechanism of PSCA monoclonal antibody is still unclear and therefore before it is officially put into clinical use, it needs further research.

3.3. Epithelial cell adhesion molecule

One of the earliest tumor-associated antigens discovered was epithelial cell adhesion molecule (EpCAM), also known as tumor-associated calcium signal transducer1 (TACSTD1). It is only expressed in normal and malignant epithelial cells therefore in mesenchymal organs, one important application of EpCAM is the diagnostic criteria of cancer cell detection. It has been proved as a homophilic calcium independent intercellular adhesion molecule playing a role in epithelial carcinogenesis including regulation of cell proliferation and cancer stemness. Through establishing mice model experiments, CAR-T cells were specifically targeted to EpCAM by lentivirus vector. EpCAM CAR-T cells can secrete cytotoxic factors and it can induce target cells to produce cytotoxin in the form of EpCAM dependent, what's more, in xenograft model growth and formation of tumor was greatly delayed by adoptive transfer of EpCAM CAR-T [19]. In the experiment, CAR-T cells do not cause any systemic damage in mice showed by safety assessment. The results showed in the experiment reflect its effectiveness and safety and it also showed the possibility of CAR-T treatment targeting to it. Now there are still few clinical data about it, and related projects are recruiting volunteers to participate, therefore, its targeted therapeutic effect on human solid tumors needs more time to verify.

4. Conclusion

CAR-T cells therapy is a kind of therapy with accuracy, speed and efficiency. Although it has not been long since it was created, its structure has undergone four generations of reform which made it can treat cancer more effectively. The commonly used CAR-T cells now consist of four parts and each part have different functions. As time goes on, its structure will be transformed more conducive as a therapy of different types of cancer in various situations.

According to the current research progress, three antigens which have prostate specific mentioned in the text show good specificity for prostate tumor cells and they all play an important role in the treatment or diagnosis of prostate cancer as therapeutic targets. What's more, CAR-T therapy experiments targeting to these three antigens are in progress and researchers have arrived at some experimental data which reflects that the existing CAR-T cells therapy can treat prostate cancer to a certain extent. Clinical trials of CAR-T cells targeting to PSMA have been conducted, which obviously reduced PSA of patients in the trial and showed excellent treatment effect for prostate cancer, but serious CRS also bring harm to patients. To PSCA, in animal trial, its monoclonal antibody can inhibit the growth of prostate cancer of tumor bearing mice, but it has no record of human clinical trials and the action mechanism of PSCA monoclonal antibody is still unclear. EpCAM was regarded as one of the first tumor-associated antigens identified and CAR-T targeting to it reflected the effectiveness and security to treat solid tumors in the establishing mice model experiments but there are little clinical data about it. In summarize, CAR-T cells therapy has the potential to be used in the treatment of prostate cancer, but at present, it cannot be directly put into treatment. There are still some unclear aspects about side effects and mechanism of CAR-T cells therapy in the treatment of prostate cancer and in order to study these clearly, more clinical trials should be conducted. More time is needed to study its treatment of prostate cancer.

For CAR-T cells therapy, there are also some problems that need to be solved in the future. CAR-T may cause cytokine release syndrome that will have a great impact on the patients' health and even threaten their life. In the follow-up study, researchers should try to minimize the harm CRS brings. And therapies were created to serve people in the world and they should be to provide more people with a way to treat diseases, so that they can live healthier and longer, but CAR -T cells therapy can't do that right now. Only a small number of people can afford the high cost of CAR-T. The main reason for this problem is that it needs to be produced by collecting patients' T cells, so it cannot be produced in large scale. To reduce the cost of CAR-T, there may be many ways, such as reforming existing technologies that we can use more advanced and more economical technologies and increasing the efficiency of cell production to reduce the cost of CAR-T cells production. Allogeneic CAR-T can be a new choice to reduce the cost. Allogeneic CAR-T cells therapy is a therapy that manufacture universal CAR-T cells by harvesting and modifying T cells from healthy donors to mass produce therapeutic CAR-T cells capable of supplying multiple patients. Allogeneic CAR-T can reduce the cost, shorten the waiting time of patients, apply to a wider range of patients, and it is not limited by the distribution of treatment centers. Therefore, it can solve main problems that autologous CAR-T cells therapy meets in the clinical applications. Although allogeneic CAR-T cells therapy has not yet been put into practical use, with the development of technology, it will be widely used in cancer treatment and solve problems that cannot be solved by existing CAR-T cells therapy.

References

- [1] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021; 71 (3): 209 - 249.
- [2] Darvin, P., Toor, S. M., Sasidharan Nair, V. et al. Immune checkpoint inhibitors: recent progress and potential biomarkers. *Exp Mol Med* 50, 1 – 11 (2018).
- [3] Feng Y, Roy A, Masson E, et al. Exposure-response relationships of the efficacy and safety of ipilimumab in patients with advanced melanoma. *Clin Cancer Res.* 2013 Jul 15; 19 (14): 3977 - 86.
- [4] Liu J, Zhong JF, Zhang X, Zhang C. Allogeneic CD19-CAR-T cell infusion after allogeneic hematopoietic stem cell transplantation in B cell malignancies. *J Hematol Oncol.* 2017; 10 (1): 35.
- [5] Ramos CA, Dotti G. Chimeric antigen receptor (CAR)-engineered lymphocytes for cancer therapy. *Expert Opin Biol Ther.* 2011; 11 (7): 855 – 73.
- [6] Hudecek, M. et al. The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. *Cancer Immunol. Res.* 3, 125 – 135 (2015).
- [7] Jensen, M. C. & Riddell, S. R. Designing chimeric antigen receptors to effectively and safely target tumors. *Curr. Opin. Immunol.* 33, 9 – 15 (2015).
- [8] Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci U S A.* 1989; 86 (24): 10024 – 8.
- [9] Finney HM, Lawson AD, Bebbington CR, Weir AN. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J Immunol.* 1998; 161 (6): 2791 – 7.
- [10] M. C. Ramello, I. Benzaid, B. M. Kuenzi, M. Lienlaf-Moreno, et al. An immunoproteomic approach to characterize the CAR interactome and signalosome. *Sci. Signal.* 12, eaap9777 (2019).
- [11] Carpenito, C., et al., Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proceedings of the National Academy of Sciences of the United States of America*, 2009. 106 (9): p. 3360 - 5.
- [12] Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther.* 2015; 15 (8): 1145 – 54.
- [13] Rajasekaran SA, Anilkumar G, Oshima E, et al. A novel cytoplasmic tail MXXXL motif mediates the internalization of prostate-specific membrane antigen. *Mol Biol Cell.* 2003; 14: 4835 - 4845.
- [14] Junghans RP, Ma Q, Rathore R, et al. Phase I Trial of Anti-PSMA Designer CAR-T Cells in Prostate Cancer: Possible Role for Interacting Interleukin 2-T Cell Pharmacodynamics as a Determinant of Clinical Response. *Prostate* (2016) 76 (14): 1257 – 70.

- [15] Narayan V, Barber-Rotenberg JS, Jung IY, et al. PSMA-targeting TGF β -insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial. *Nat Med.* 2022; 28 (4): 724 – 34.
- [16] Reiter, R. E., Gu, Z., Watabe, T., et al. Prostate stem cell antigen: A cell surface marker overexpressed in prostate cancer. *Proc. Natl. Acad. Sci. USA* 1998, 95, 1735 – 1740.
- [17] Reiter, R. E. et al. Co-amplification of prostate stem cell antigen (PSCA) and MYC in locally advanced prostate cancer. *Genes Chromosomes Cancer* 27, 95 – 103 (2000).
- [18] Saffran DC, Raitano AB, Hubert RS, et al. Anti-PSCA mAbs inhibit tumor growth and metastasis formation and prolong the survival of mice bearing human prostate cancer xenografts [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2001, 98 (5): 2658 – 2663.
- [19] Bing-Lan Zhang, Dan Li, You-Ling Gong, et al. Preclinical Evaluation of Chimeric Antigen Receptor–Modified T Cells Specific to Epithelial Cell Adhesion Molecule for Treating Colorectal Cancer. *Human Gene Therapy*. Apr 2019. 402 - 412.